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Protection of *Brassica* Seedlings against Downy Mildew and Damping-off by Seed Treatment with CGA 245704, an Activator of Systemic Acquired Resistance

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Abstract: Control of seedling diseases is a major priority in many crop systems. Seed treatments that induce systemic resistance after seedling emergence may be an ideal way to provide protection against disease during the establishment of the crop. CGA 245704, a chemical activator of systemic acquired resistance, was tested as a seed treatment against two *Brassica* diseases with contrasting infection biologies, the airborne downy mildew pathogen, *Peronospora parasitica*, and the soilborne fungus, *Rhizoctonia solani*. Seeds of two *Brassica* spp. were either imbibed with various concentrations of the compound or imbibed and then dried. Both the imbibition treatment alone and the imbibition treatment followed by seed drying had a significant effect on the sporulation intensity of *P. parasitica* for all concentrations of the compound used, whereas the imbibition treatment provided some control of damping-off caused by *R. solani*, with the degree of control being highly dependent on the concentration applied to the seed. Seed treatment with the plant activator CGA 245704 might therefore simultaneously control several seedling diseases, thereby providing a novel option for management of these diseases. © 1998 SCI.

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Key words: benzothiadiazole; seed treatment; seedling diseases; systemic acquired resistance; CGA 245704

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1 INTRODUCTION

Control of seedling diseases is a high priority in many crops, as early infection by pathogens may kill the plant, leading to poor crop establishment or even complete loss. Applying fungicides as soil drenches, sprays or in irrigation water can reduce losses, but the preferred economic option is to treat seeds. Ideally a compound applied to seed may provide protection during germination, emergence and the early establishment phase of the crop.

Systemic acquired resistance (SAR) is a natural plant defence response in which exposure to pathogens causing necrosis induces enhanced resistance to subsequent infection.[1] SAR can also be induced by certain chemicals, including salicylic acid and analogues.[2,3] This observation has been used to develop SAR as a novel chemical control strategy.[4] Recently, Ciba-Geigy announced CGA 245704 (*S*-methyl benzo[1,2,3] thiadiazole-7-carbothioate), a plant defence activator which can be used to confer protection against a wide spectrum of plant pathogens.[5–7] This compound has a different mode of action from conventional fungicides and is therefore recommended for integrated control of a variety of plant diseases.

The current study assessed the effect of seed treatment with CGA 245704, on early infection of brassica seedlings by two fungal pathogens with contrasting infection biologies. *Peronospora parasitica* (Pers. ex Fr.) Fr. is a biotrophic foliar pathogen causing downy mildew, and is typically spread by airborne conidia.[8] The disease is particularly damaging in *Brassica oleracea* seedlings raised in glasshouses for transplanting into the field; fungicides are used routinely to protect seedlings during this vulnerable phase.[9] Problems have arisen, however, with the development of resistance to phenylamide fungicides[10] in the *B. oleracea*-adapted population of the pathogen.[11] There is a need, therefore, for alternative control strategies. *Rhizoctonia solani* Kühn is a necrotrophic soilborne pathogen which infects roots and the stem-base,[12] causing pre- and post-emergence damping-off, root rot and basal stem rot, especially in oilseed rape. Seed-application with fungicides is the only effective control method, although biological alternatives are being evaluated.[13] Two CGA 245704 treatments were compared, imbibition of seeds followed directly by sowing, or imbibition of seeds which were then dried prior to sowing. The latter mode of application would be of particular utility as the compound could be applied by seed suppliers rather than by the grower.

2 MATERIALS AND METHODS

2.1 Plants and pathogen isolates

For experiments with downy mildew, *P. parasitica*, a single spore isolate from Oregon, USA (OR93.cab-ss1), originating from cabbage, *B. oleracea* L., was used. The cabbage cv. Scanner F_1 (L. Dæhnfeldt, A/S) was the experimental host.

The isolate of *R. solani*, obtained from oilseed rape (*B. napus* L.), was kindly supplied by Dr S. Rossall, University of Nottingham, UK. Oilseed rape cv. Express (Twyfords, UK) was used as the experimental host.

2.2 Seed treatment

Two treatments were compared:

A. Seed imbibition (I): Seeds of the two *Brassica* spp. were imbibed for 24 h in a suspension of CGA 245704 made up in sterile distilled water (SDW), then sown directly.
B. Seed imbibition followed by drying (I + D): Imbibition was carried out as above, and seeds were then air-dried on filter paper at room temperature for 24 h prior to sowing.

The following concentrations of activator were tested: 0 (SDW control), 10, 20, 30 and 50 mg litre^{-1}.

2.3 Downy mildew experiment

Seeds of *B. oleracea* cv. Scanner were sown in 6 × 6 cm pots in soil-less compost (Levington F2, UK) at a rate of 16 seeds per pot. The pots were arranged in a split-plot design with three replications. The seed treatments were applied as main plot treatments with the different concentrations of activator applied to subplots. The seedlings were raised under greenhouse conditions and thinned to 10 per pot. Nine-day-old seedlings were inoculated with a conidial suspension of *P. parasitica* (2×10^4 conidia ml^{-1}).[11] A 10-μl droplet was applied with a pipette to the centre of each cotyledon. The pots were placed in propagators on wetted filter paper in a Fisons growth cabinet (Model 600 H) at 15°C with a 12-h photoperiod (130 μmol m^{-2} s^{-1} inside the propagators).

Eight days after inoculation, each seedling was assessed for sporulation intensity on a scale from 0 to 5. (0 = no sporulation, 1 = sporulation confined to point of infection and not exceeding 3–4 conidiophores, 2 = sporulation covering inoculation site, 3 = sporulation covering more than inoculation site, but less than 4, 4 = sporulation covering the whole cotyledon, 5 = heavy sporulation on the whole cotyledon). Additionally, the host response was described by the absence or presence of dark flecking at the inoculation site.

Data were analysed using analysis of variance (SAS/STAT version 6.08, SAS Institute Inc., Cary, NC, USA).

2.4 *Rhizoctonia* experiment

Inoculum cultures of *R. solani* were prepared by seeding

100-ml aliquots of Czapek-Dox plus V8 liquid medium in 250-ml conical flasks with agar blocks (1 cm^3) cut from a plate culture of the fungus. Flasks were incubated in the dark at 25°C for three weeks. Mycelial mats were harvested by filtration, blotted dry on sterile paper towels, and macerated for 2 min in an electric blender (Roclab; medium speed setting). The macerate (25·5 g) was mixed thoroughly with 2·55 kg of heat-sterilized compost and the resulting mixture was dispensed in 200-ml volumes into 6 × 6 cm pots. Control pots contained uninfested compost.

Seeds of oilseed rape (*B. napus*) cv. Express were sown onto the surface of the compost at a rate of 20 seeds per pot. Pots were maintained at 16°C in a growth cabinet with a 12-h photoperiod as described in Section 2.3. Three replicate pots were used for each combination of treatment and activator concentration.

Germination (emergence) was assessed seven days after sowing. Symptomless seedlings (i.e. those without any detectable browning at the base of the hypocotyl) were counted at seven and 14 days after sowing.

Both the number of seeds which germinated and the number of seedlings without symptoms were assumed to be binomially distributed so that generalized linear models with a logit link were fitted to the data. For the symptom data the full model, including all main effects and interactions between the factors, was fitted to check that there was no evidence of over-dispersion. Logistic curves were then fitted to the symptom data, again assuming binomially distributed responses. The response curve fitted was:

$$y = (1 - pi) - \frac{(1 - pi - pc)}{1 + \left(\frac{dose}{e^{m}}\right)^{b}} \quad (1)$$

a parameterization of the standard logistic which facilitates a log-transformation of the doses where a zero dose is included (Fig. 1). The parameter *pc* is the proportion of symptomless seeds with no dose, while *pi* is the proportion which shows symptoms at very high doses.

After fitting separate curves to each of the four combinations of treatment (I/I + D) and time (7/14), parallel curve analysis was used to reduce the number of parameters.

3 RESULTS

3.1 Effects on growth of seedlings

Emerging seedlings from CGA 245704-treated seed had smaller and darker green cotyledons than control seedlings imbibed in SDW. This tendency held throughout the experiments and was more pronounced at high activator concentrations.

3.2 Effects on downy mildew infection

Sporulation was first observed on control seedlings four days after inoculation. At this time, all activator-treated

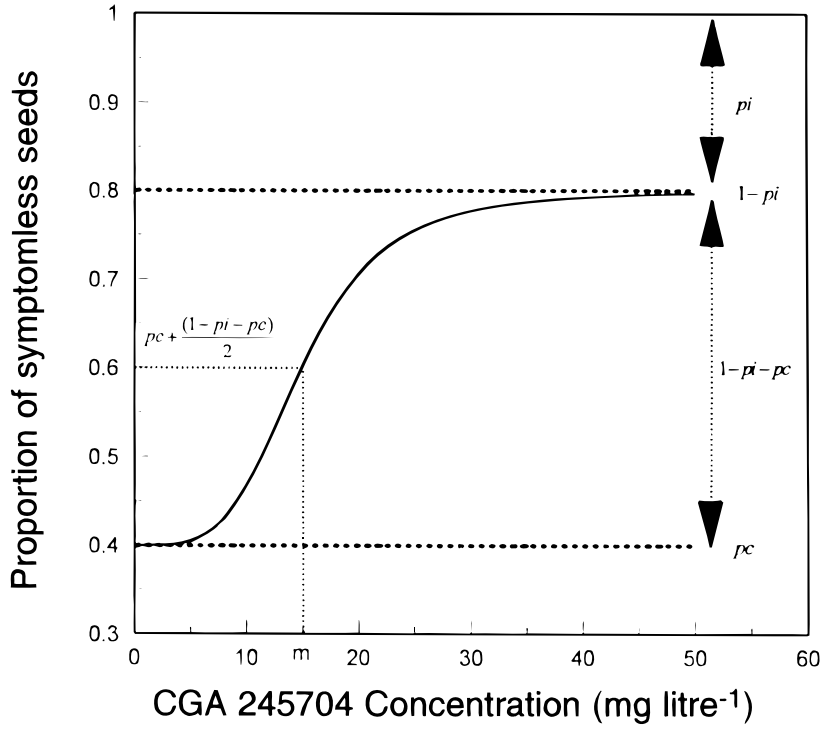

Fig. 1. Diagram to illustrate the interpretation of parameters in eqn (1).

TABLE 1
Downy Mildew Sporulation Intensity Eight Days after Inoculation of Cotyledons of Nine-Day-Old Seedlings of *Brassica oleracea* cv. Scanner, sown after Seed Treatment with Different Concentrations of CGA 245704

CGA concentration (*mg litre*$^{-1}$)	*Mean sporulation intensity*
0	4·65
10	0·65
20	0·57
30	0·83
50	0·78

SED = 0·214, 16 df, $LSD_{0·05}$ = 0·45.

seedlings exhibited a dark flecking reaction at the inoculation site, and no sporulation was visible.

Analysis of variance on the sporulation data assessed eight days after inoculation indicated a significant main effect of treatment ($P = 0·042$) and of concentration ($P < 0·001$) but no interaction between these. The I + D treatment showed less sporulation (intensity = 1·31) than the I treatment alone (intensity = 1·68). All concentrations of the compound resulted in significantly less sporulation than the control treatment, while differences between concentrations were not significant (Table 1).

Microscopic examination of cleared and stained cotyledons eight days after inoculation revealed extensive growth of intercellular hyphae forming haustoria in mesophyll cells, with only occasional host cell necrosis in control tissues, while in activator-treated tissues there was restricted hyphal growth with widespread host cell necrosis beneath the inoculation site.

3.3 Effects on infection by *Rhizoctonia*

3.3.1 Germination data

The germination rate of seeds in uninfested compost was 100% seven days after sowing. No phytotoxic effects of the activator treatment were observed.

Fitting the generalized linear model revealed strong evidence of an interaction ($P = 0·004$), in addition to main effects of treatment and concentration, on the proportion of seeds which germinated. That is, the effect of

TABLE 2
Number of Germinated *Brassica napus* Seeds (out of 20 per Module) treated with Different Concentrations of CGA 245704 Seven Days after Sowing in *Rhizoctonia*-Infested Compost

	CGA 245704 concentration (*mg litre*$^{-1}$)				
Treatment	*0*	*10*	*20*	*30*	*50*
I[a]	16, 18, 20	20, 20, 20	20, 20, 20	20, 20, 20	20, 20, 20
Mean (%)[b]	90 (±3·87)	100 (0)	100 (0)	100 (0)	100 (0)
I + D	9, 14, 8	7, 11, 6	10, 12, 11	19, 11, 16	13, 10, 13
Mean (%)[b]	51·7 (±6·45)	40 (±6·32)	55 (±6·42)	76·7 (±5·46)	60 (±6·32)

[a] I: Seed imbibition, I + D: seed imbibition followed by seed drying.
[b] Approximate standard errors in parentheses.

TABLE 3
Number of Symptomless *Brassica napus* Seedlings (out of 20 per Module) treated with Different Concentrations of CGA 245704 Seven and 14 Days after Sowing in *Rhizoctonia*-Infested Compost

		CGA 245704 concentration (*mg litre*$^{-1}$)				
Treatment	*Day*	*0*	*10*	*20*	*30*	*50*
I[a]	7	10, 12, 12	16, 12, 14	16, 15, 16	18, 17, 16	20, 20, 18
	14	7, 6, 7	10, 11, 11	14, 13, 12	14, 16, 13	17, 18, 15
I + D	7	4, 6, 5	4, 8, 2	7, 6, 7	10, 7, 7	6, 8, 8
	14	2, 4, 3	2, 8, 2	6, 7, 6	10, 6, 6	6, 7, 8

[a] I: Seed imbibition, I + D: seed imbibition followed by seed drying.

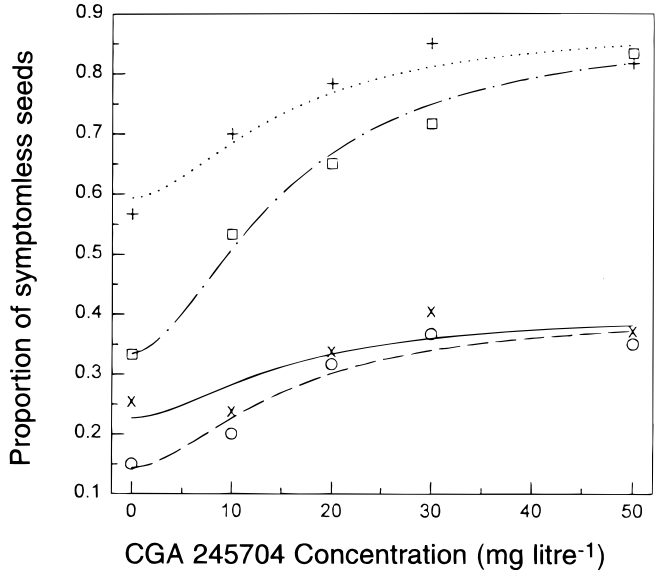

Fig. 2. Mean proportion of symptomless *Brassica napus* seeds, either imbibed or imbibed then dried, with fitted curves, at various concentrations of CGA 245704, seven or 14 days after sowing in *Rhizoctonia solani*-infected compost. (×, ———) I + D, 7, (○, – – – –) I + D, 14, (+, · · · · · · ·) I, 7, (□, –·–·–·) I, 14.

concentration on the percentage germination was different in I and I + D seeds (Table 2). The proportion which germinated was high at all doses for the imbibed treatment but was lower and more variable when the seeds were imbibed and then dried.

3.3.2 *Symptom data*

The number of seeds which germinated to give symptomless seedlings seven and 14 days after sowing is shown in Table 3, and plotted as a proportion of the original 20 seeds in Fig. 2. From the initial model, with all of the parameters in eqn (1) estimated for each of the four combinations of treatment and time, it was found that a simplified form could be fitted with only two upper asymptotes. *Pi*, the proportion of seeds which either failed to germinate or showed browning symptoms at a very high dose, was 0·12 in seeds which were imbibed, but increased to 0·60 in seeds which were imbibed and then dried. *Pi* was unaffected by time—at high doses the proportion of symptomless seeds did not change from seven to 14 days.

In contrast, the incidence of disease in the absence of compound (*pc*) varied with both seed treatment and time. Imbibed seeds which were directly planted showed much higher rates of emergence and less hypocotyl browning than imbibed and dried seeds. There was an increase in disease with time with both seed treatments.

Parameter *b*, associated with the 'relative' response rate, and parameter *m*, the dose at which the number of symptomless seeds is $(1 - pi - pc)/2$, halfway between the lower and upper asymptotes, may be assumed constant over all four of the treatment combinations.

The severity of damping-off was reduced by both treatments, but after 14 days the majority of seedlings in the I + D treatment showed symptoms of infection by *R. solani*. This contrasted with the I treatment which afforded relatively good protection after 14 days, especially at the higher concentrations.

The parameters of the final fitted model are presented in Table 4.

TABLE 4

Parameter Estimates for Eqn (1) fitted to the Proportion of Oilseed Rape Seeds, either Imbibed in CGA 245704 or Imbibed and then Dried, which Germinated and remained Symptomless after Seven or 14 Days' Exposure to *Rhizoctonia solani*-Infested Compost

	Estimate	*Standard error*
$pc_{(I+D,\ 7)}$	0·2269	0·0455
$pc_{(I+D,\ 14)}$	0·1432	0·0401
$pc_{(I,\ 7)}$	0·5936	0·0531
$pc_{(I,\ 14)}$	0·3344	0·0564
$pi_{(I+D)}$	0·5984	0·0501
$pi_{(I)}$	0·1204	0·0716
ED_{50} (e^m)	15·394	4·233
b	1·740	0·738

4 DISCUSSION

Induced resistance through activation of plant natural defence pathways has been shown to be effective against a wide variety of plant pathogens.[3] Current models of SAR assume acropetal translocation of a soluble signal

molecule, with potentiation of resistance in aerial tissues.[4] Hence, most available data concern effects on foliar pathogens such as leaf blights, powdery and downy mildews. Much less has been published on effects on pathogens infecting basal tissues and roots. Good control of downy mildew, assessed as reduced sporulation, was obtained with both treatments at all concentrations of the compound used. Previous work has shown that infection of *Arabidopsis*[14] and *Brassica*[15] sp. by *P. parasitica* is reduced by treatment with chemical elicitors of SAR, but this is the first report of effective induction by treating seed. Interestingly, significantly better control was found with the dried seed treatment (I + D) than when imbibed seed was directly sown (I). Further testing is required to determine the effect of seed storage after drying on efficacy, since adequate persistence would make it possible for seed suppliers to treat large quantities of seed prior to distribution to growers.

Contrasting results were obtained in the *R. solani* infection trial, where superior control was obtained when imbibed seed was planted directly. However, this was not due simply to differences in the efficacy of the compound in the two treatments, as major differences were also seen in the water-treated controls. It seems likely that the imbibed seed was able to germinate and establish more effectively in the presence of the pathogen than dry seeds, possibly due to initial effects on the rate of seedling growth. Subsequently, an increasing proportion of non-treated seedlings developed symptoms of infection, while activator treatment provided significant protection, even at the high inoculum rate used in the experiment. With the dried (I + D) seeds, some benefit was observed, but overall disease severity after 14 days was high.

The current experiments confirm that a chemical activator of SAR, applied to seed, gives good protection against foliar downy mildew in *Brassica* seedlings. As downy mildew poses a serious threat to young *Brassica* seedlings, the current results suggest that CGA 245704 used as a seed treatment would be a valuable addition to existing strategies for integrated control of the disease. The novel mode of action would be a further advantage in situations where fungicide insensitivity, for instance to acylalanides,[10] is known to be a problem. Some control of a damping-off pathogen may also be achieved. Seed treatment with defence activators might therefore simultaneously control several seedling diseases, thereby providing a novel option for management of early infections.

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